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Shearing of synovial fluid activates latent TGF-β

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SUMMARY

Objective: TGF-β is synthesized in an inactive latent complex that is unable to bind to membrane receptors, thus unable to induce a cellular biological response until it has been activated. In addition to activation by chemical mediators, recent studies have demonstrated that mechanical forces may activate latent TGF-β *via* integrin-mediated cellular contractions, or mechanical shearing of blood serum. Since TGF-β is present in synovial fluid in latent form, and since normal diarthrodial joint function produces fluid shear, this study tested the hypothesis that the native latent TGF-β1 of synovial fluid can be activated by shearing.

Design: Synovial fluid from 26 bovine joints and three adult human joints was sheared at mean shear rates up to 4000 s^{-1} for up to 15 h.

Results: Unsheared synovial fluid was found to contain high levels of latent TGF-β1 (4.35 ± 2.02 ng/mL bovine, 1.84 ± 0.89 ng/mL human; mean ± radius of 95% confidence interval) and low amounts (<0.05 ng/mL) of the active peptide. Synovial fluid concentrations of active TGF-β1 increased monotonically with shear rate and shearing duration, reaching levels of 2.64 ± 1.22 ng/mL for bovine and 0.60 ± 0.39 ng/mL for human synovial fluid. Following termination of shearing, there was no statistical change in these active levels over the next 8 h for either species, demonstrating long-term stability of the activated peptide. The unsheared control group continued to exhibit negligible levels of active TGF-β1 at all times.

Conclusions: Results confirmed the hypothesis of this study and suggest that shearing of synovial fluid might contribute an additional biosynthetic effect of mechanical loading of diarthrodial joints.

Introduction

Transforming growth factor beta (TGF-β) is a ubiquitous multifunctional cytokine that modulates the proliferation, differentiation and extracellular matrix (ECM) production of various biological tissues[1]. While most cells express TGF-β receptors[2,3], there has been particular interest in its role in the development and maintenance of diarthrodial joints due to the high native levels of TGF-β in cartilage[4–6] and the surrounding synovial fluid[7–9]. Traditional investigations into the regulation of TGF-β signaling in cartilage have focused primarily on characterizing its synthesis, and its influence on the metabolic activity of chondrocytes. These studies have typically shown that TGF-β can greatly impact biosynthesis by chondrocytes[10–14] and that its production is regulated by a variety of mechanical[8,15] and chemical factors[5].

In cartilage, as in other tissues, TGF-β is synthesized in an inactive latent complex that is unable to bind to membrane receptors, thus unable to induce a cellular biological response until it has been activated. In this latent complex, the mature TGF-β 25 kDa peptide is linked non-covalently to a 70 kDa latency associated peptide (LAP), together forming the small latent complex (SLC). This complex may be disulfide-bonded to a latent TGF-β binding protein (LTBP, ~180 kDa), constituting a configuration termed the large latent complex (LLC)[16,17]. In growth plate cartilage, both SLC and LLC are secreted by chondrocytes; the LLC can bind to the ECM *via* the LTBP[18,19]. In synovial fluid, latent TGF-β likely resides in an unbound soluble form. In order to elicit a biological response, the mature TGF-β peptide must first be released from the LAP in a process termed TGF-β activation[16,17]. Although this process is essential for TGF-β signaling, only a few studies have investigated potential activation mechanisms in cartilage and synovial fluid.

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Investigations into potential release mechanisms for other tissues have shown that TGF-β activation can occur through a variety of chemical mediators, including proteases such as plasmin[20] and matrix metalloproteinases (MMPs)[21], thrombospondin (TSP)[22], and reactive oxygen species[23]. It is generally believed that activation mechanisms are tissue-specific and for any given tissue, several mechanisms may be involved[17]. In arthritic synovial fluid, it has been suggested that metalloproteinases may be responsible for the activation of TGF-β[24]. Studies in cartilage have shown that matrix-bound latent TGF-β can be activated by stromelysin-1 (MMP-3)[25] and collagenase-3 (MMP-13)[26] released from cellular matrix vesicles during endochondral ossification, and by transglutaminase in aging articular cartilage[27].

In addition to activation by chemical mediators, recent studies have demonstrated that mechanical forces may also activate latent TGF-β[28]. This concept was originally demonstrated in a study by Wipff *et al.*[29], where integrin-mediated cellular contractions were shown to pull on matrix-bound latent TGF-β to induce activation. Subsequently, in a study by Ahamed *et al.*[30], mechanical shearing of blood serum was shown to activate soluble TGF-β. It is believed that these mechanically-induced activation mechanisms may play an important role in physiologic processes such as wound healing.

Although this mechanism has only been described in a few tissue systems, mechanically-induced TGF-β activation may potentially play an important physiologic role in any tissue that contains latent TGF-β and is subjected to large repetitive mechanical loads. In the case of diarthrodial joints, in addition to the well recognized cyclic compression experienced by cartilage, synovial fluid is subjected to reciprocal shearing between sliding articular surfaces. During routine physiologic joint motion, the shear rate of synovial fluid can reach levels more than 10 times those achieved in arterial blood flow[31].

Based on this understanding, the objective of this experimental study was to test the hypothesis that shearing of synovial fluid can activate its native latent TGF-β. To test this hypothesis, synovial fluid was subjected to continuous *in vitro* shearing at rates that approach physiologic levels, and the active concentration of TGF-β1 was measured over time. Mature and immature bovine synovial fluid was used to explore the dependence of activation on fluid shear rate and adult human synovial fluid was used to confirm the physiologic relevance of this mechanism. Furthermore, an additional test was conducted to assess the stability of this freshly activated TGF-β based on the traditional understanding that TGF-β has a short *in vivo* half-life due to its interactions with a variety of non-specific binding proteins[32,33]. Since recombinant latent TGF-β is commercially available in the SLC, shear activation of the exogenous SLC in synovial fluid was also investigated.

Methods

Materials

Active TGF-β strongly binds and adheres to plasticware[34] due to its high degree of hydrophobicity, making it potentially difficult to accurately measure its concentration *in vitro*. Therefore, only materials which exhibited negligible binding to TGF-β were used in this study (see Supplementary Data). To this end, synovial fluid was sheared inside Tygon tubing (Saint-Gobain Corporation, www.tygon.com) and collected and stored in silanized Eppendorf tubes (www.eppendorf.com) before and after testing. Synovial fluid was aspirated from the carpometacarpal joints of 2-month-old immature ($N = 8$ joints) and 6-year-old adult ($N = 22$ joints) bovines supplied by a local slaughterhouse (Green Village Packing Co., NJ). After acquisition, all bovine specimens were decellularized through centrifugation (3000*g*, 20 min). For each source, synovial fluid samples were generated by pooling the fluid from two individual joints, producing four samples for immature synovial fluid and 11 samples for mature synovial fluid. Each sample was divided into multiple 1.5 mL aliquots and stored at −30°C until testing.

Human synovial fluid was obtained and frozen from three autopsy donors (ages 57, 59, and 61 years, 4 mL per donor), within 8 h postmortem through the National Disease Research Interchange (NDRI). These specimens were filtered through a polyester mesh (100 μm) to remove debris, but were not subjected to centrifugation.

At the completion of testing, all synovial fluid samples were collected, flash frozen in a bath of ethanol with dry ice, and stored at −30°C until subsequent ELISA analysis.

Synovial fluid shearing: temporal response

Synovial fluid was injected into Tygon tubing (R-3603, 1/32" ID) and subjected to continuous circulation at a prescribed flow rate (see below) with a peristaltic pump or maintained uncirculated as a control group. The process of circulating a viscous fluid in a tube produces shearing as a result of frictional interactions with the inner tube wall. For a Newtonian fluid, the shear rate increases linearly along the radial direction of the tube, starting from zero at the centerline. Though synovial fluid is not a Newtonian fluid, an estimate of the mean shear rate was obtained under the simplifying assumption of Newtonian behavior. Accordingly, the pump speed was prescribed to maintain mean shear rates based on Hagen–Poiseuille law. Adult bovine synovial fluid was subjected to shear rates of 0, 600, 1700, or 4000 s^{-1}, each for a duration of 2, 7, and 15 h. This test was conducted with four synovial fluid samples for each group, using 12 aliquots (four shear rates × three time points) from each sample.

Immature bovine synovial fluid was subjected to shear at 4000 s^{-1} or maintained uncirculated for a duration of 2, 7, and 15 h. This test was conducted with four synovial fluid samples for each group, using six aliquots (two shear rates × three time points) from each sample.

For human synovial fluid testing, each donor specimen was subjected to shear at 3750 s^{-1} or maintained uncirculated (2 mL each). Here, small aliquots were collected immediately before, then after 2 and 8 h of testing.

Though experiments were conducted at room temperature, continuous shearing of synovial fluid over several hours produced an increase in its temperature, especially at the higher shear rates. Measurements determined that for all bovine synovial fluid samples, the peak temperature never exceeded 37°C. All control (unsheared) samples were maintained at 37°C. For human synovial fluid testing, the temperature approached 41°C for part of the test. However, preliminary studies in adult bovine synovial fluid ($n = 3$ samples each at 37, 45, and 56°C) showed that this slightly elevated temperature for up to 8 h does not produce any alterations to active and total TGF-β levels (Fig. 1).

Synovial fluid shearing: SLC

It has been shown that chondrocytes secrete latent TGF-β in the SLC form[18,19], suggesting it may also be present in synovial fluid. Since recombinant SLC is readily available, an additional test was conducted to independently assess whether it will similarly undergo shear-induced activation in synovial fluid. Here, adult bovine synovial fluid was supplemented with 35 ng/mL of human recombinant SLC-configured latent TGF-β1 (R&D Systems). This concentration of exogenous latent TGF-β is an order of magnitude higher than the endogenous levels in synovial fluid (see Results) allowing for the assessment of exogenous SLC latent TGF-β1

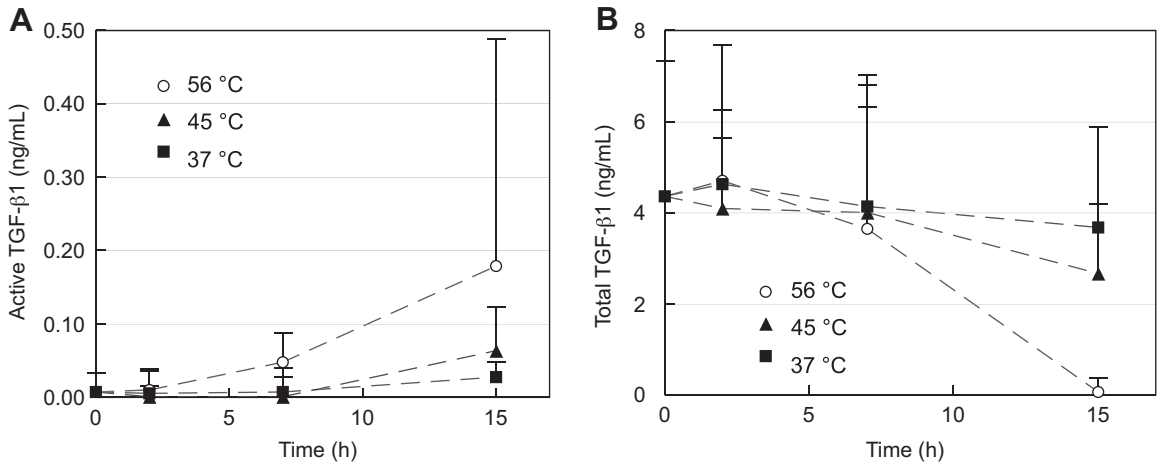

Fig. 1. Effect of temperature on the stability of latent TGF-β1 in synovial fluid. Concentration of (A) active and (B) total TGF-β1 in adult bovine synovial fluid after exposure to various temperatures for up to 15 h. All TGF-β1 is initially in the latent form and is stable at all temperatures for up to 7 h. After 15 h at 56°C, all TGF-β1 is degraded except for a small fraction that remains in the active form ($n = 3$ sample per temperature group; mean ± radius of 95% confidence interval).

activation. This supplemented synovial fluid was continuously sheared at 1700, 4000 s^{-1} or maintained unsheared for 0.5, 2, or 7 h. This test was conducted with three samples for each group, using nine aliquots (three shear rates × three time points) from each sample.

Synovial fluid shearing: protease inhibition

An additional test was conducted to preclude the action of proteases in activating latent TGF-β. Adult bovine synovial fluid was either supplemented with protease inhibitors (Complete Cocktail 1× w/EDTA, Roche) or maintained as an untreated control group. Both groups were subjected to continuous shearing at 4000 s^{-1} for 7 h. This test was conducted with four samples for each group, using one aliquot (one shear rate × one time point) from each sample.

Stability of activated TGF-β

A final test was conducted to explore the stability of TGF-β1 after its activation in synovial fluid.

Sheared synovial fluid from the previously completed tests (adult bovine synovial fluid 15 h at 4000 s^{-1}, human synovial fluid 8 h at 3750 s^{-1}, SLC supplemented adult bovine synovial fluid 7 h at 4000 s^{-1}; three samples from each) was transferred to a silanized Eppendorf tube and maintained unsheared for an additional 8-h period at 37°C. During this period, a small aliquot from each group was collected after 0, 2, and 8 h.

Enzyme-linked immunosorbant assay (ELISA)

Tested synovial fluid aliquots were thawed and the concentration of active TGF-β1 was measured in duplicate with a TGF-β1 ELISA kit (R&D Systems), using recombinant human TGF-β1 as standards. Only active TGF-β1 can be detected by this ELISA kit. Therefore, in order to also measure total TGF-β1 in synovial fluid, samples were subsequently subjected to an acid activation treatment consisting of acidification for 15 min with 20% of 1 N HCl, followed by neutralization with an equal volume of 1.2 N NaOH/0.5 M HEPES, and a second ELISA analysis. This is a routine procedure for activating all latent TGF-β1 in biological samples, allowing for the measurement of their total TGF-β1 content[18,35]. This measure accounted for the low but non-negligible levels of latent TGF-β1 in the R&D Systems serum-based Reagent Diluent.

Preliminary tests suggested that fluid samples of high viscosity exhibit suppressed TGF-β levels when assayed through ELISA. Although synovial fluid is highly viscous, this issue was not explicitly addressed in previous reports on TGF-β concentrations in synovial fluid. Therefore, a preliminary test was conducted to assess the effect of diluting synovial fluid samples on mitigating this effect. The TGF-β1 content of sheared adult bovine synovial fluid (2 h, 4000 s^{-1}) was measured after dilution in Reagent Diluent at dilution factors (DF) of 1, 2.5, 5, 10, 20, 40, 80. At each dilution, measured ELISA concentration was multiplied by the DF and normalized to the value obtained at 80 DF. Results demonstrate that diluting synovial fluid increases its apparent TGF-β1 concentration (Fig. 2). At higher dilutions, this increase levels off to the actual concentration in synovial fluid. In effect, undiluted synovial fluid yields a measure of only 20% of this actual concentration. Subsequently, a minimum DF of 10 was adopted for all ELISA measurements in this study, due to its yield of 80% of the actual TGF-β1 concentration.

Statistical analysis

In each study, one statistical analysis was performed on the concentrations of active TGF-β1 and another on the total

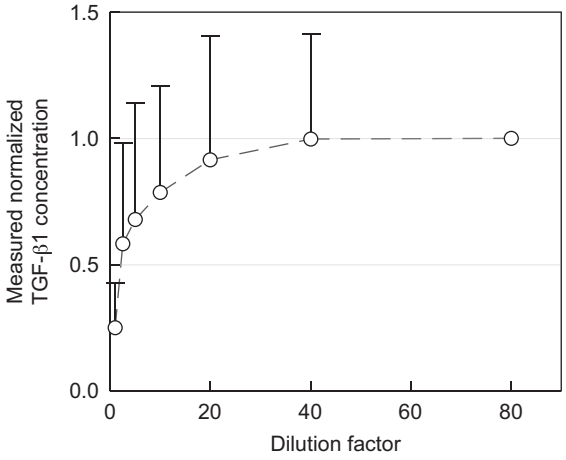

Fig. 2. Apparent concentration of shear-activated TGF-β1 in synovial fluid after various amounts of dilution (mean ± radius of 95% confidence interval). Concentrations are corrected for the DF then normalized to the value evaluated at the highest dilution.

concentration of TGF-β1. For the temporal response study, a two-way analysis of variance (ANOVA) was performed, with $\alpha = 0.05$ and statistical significance set at $P < 0.05$, to determine the effects of shearing rate and testing duration, using repeated measures for both factors since aliquots from the same sample were used for each cell ($n = 4$ samples in each cell). For the adult bovine synovial fluid, a *post-hoc* power analysis for this design indicated an achieved power of nearly 100% for detecting differences in active TGF-β1 concentrations and 11% for detecting differences in total TGF-β1 concentrations. For the protease inhibition study, a paired two-tailed Student's *t* test was used, with $\alpha = 0.05$, to detect differences between the two tested groups, with aliquots from the same sample used in each group ($n = 4$ samples). A *post-hoc* power analysis indicated an achieved power of 14% for detecting differences. Two-way ANOVA was also used for the SLC study and the study on the stability of activated TGF-β1.

Results

Synovial fluid shearing: temporal response

Prior to shearing, high levels of total TGF-β1 were measured in synovial fluid (4.35 ± 2.02 ng/mL adult bovine, 3.66 ± 1.12 immature bovine, 1.84 ± 0.89 ng/mL human; mean ± radius of 95% confidence interval). However, at all time points under control (unsheared) conditions, negligible TGF-β1 levels were detected in synovial fluid (0.01 ± 0.03 ng/mL in adult bovine, 0.05 ± 0.2 ng/mL in immature bovine, and 0.03 ± 0.03 ng/mL in human), indicating that TGF-β1 was predominantly in a latent form. Similar levels of active TGF-β1 (0.01 ± 0.02 ng/mL, $n = 3$ samples) were detected in adult bovine synovial fluid before freezing as well, indicating that freezing had no effect on activation. Under continuous shearing, active TGF-β1 in adult bovine synovial fluid was observed to increase significantly ($P < 0.001$) with time and shearing rate; adult bovine synovial fluid reached a value of 2.64 ± 1.22 ng/mL [Fig. 3(A)] and immature bovine synovial fluid reached 2.73 ± 0.56 ng/mL [Fig. 4(A)] after 15 h at the highest shear rate (4000 s^{-1}). Similarly, for human synovial fluid, shearing at 3750 s^{-1} activated 0.60 ± 0.39 ng/mL of TGF-β1 after 8 h [Fig. 5(A)]. Furthermore, for all groups and shearing conditions, the concentration of total TGF-β1 did not change over time ($P = 0.95$ adult bovine; $P = 0.88$ immature bovine; $P = 0.94$ human, Figs. 3(B), 4(B) and 5(B)).

Synovial fluid shearing: SLC

For this experiment, exogenous SLC content was determined by measuring total content and subtracting native levels (average concentrations assessed from prior tests), yielding 36.9 ± 2.5 ng/mL for the initial (unsheared) content. Exogenous active content was similarly assessed. When reporting results, total and active exogenous TGF-β1 concentrations are expressed as a fraction of this initially supplemented exogenous concentration. Initially, and in the absence of shearing, exogenous TGF-β1 was nearly entirely in its latent form, as indicated by an active fraction of 0.012 ± 0.011. The active level of exogenous TGF-β1 increased significantly with time and shearing rate [Fig. 6(A)], reaching an active fraction of 0.67 ± 0.61 after 7 h at 4000 s^{-1}. Furthermore, under all shearing conditions, the concentration of total TGF-β1 did not change over time [$P = 0.96$, Fig. 6(B)].

Synovial fluid shearing: protease inhibition

The inhibition of protease activity in synovial fluid had no effect on the shear-induced activation of latent TGF-β. No statistical difference in activated concentrations between protease-inhibitor treated and control samples was observed in adult bovine synovial fluid after 7 h of shearing ($P = 0.36$, Table I).

Stability of activated TGF-β

For all groups (human, adult bovine, and SLC supplemented bovine synovial fluid), upon cessation of shearing, no statistical change to active TGF-β levels was observed over a subsequent 8 h period ($P = 0.91$ adult bovine; $P = 0.34$ human; $P = 0.98$ SLC supplemented; Fig. 7), indicating that shear-induced activated TGF-β is stable in synovial fluid.

Discussion

It has been well established that TGF-β is an important regulator of the development and maintenance of articular cartilage[36]. However, as with most biological tissues, the TGF-β present in diarthrodial joints is produced as a latent complex that must be activated in order to induce a biological response[7,18]. Only a few studies have characterized the potential physiologic mechanisms by which this activation occurs, focusing on chemical mediators. The results of this study demonstrate two new and important findings: (1) mechanical shearing of synovial fluid can activate substantial concentrations of latent TGF-β (Figs. 3–6); and (2) these activated TGF-β concentrations remain stable in synovial fluid (for 8 h at least, Fig. 7). These results strongly suggest that *in vivo* shearing of synovial fluid through physiologic joint motion can

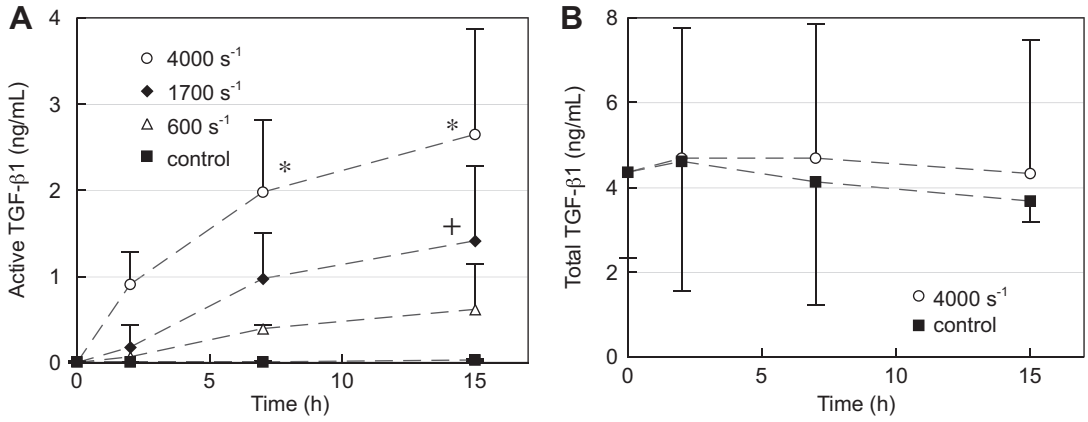

Fig. 3. Concentration of (A) active and (B) total TGF-β1 in adult bovine synovial fluid undergoing continuous circulation at various shear rates (mean ± radius of 95% confidence interval). *$P < 0.001$, +$P = 0.002$ indicate statistical increase above corresponding control time point.

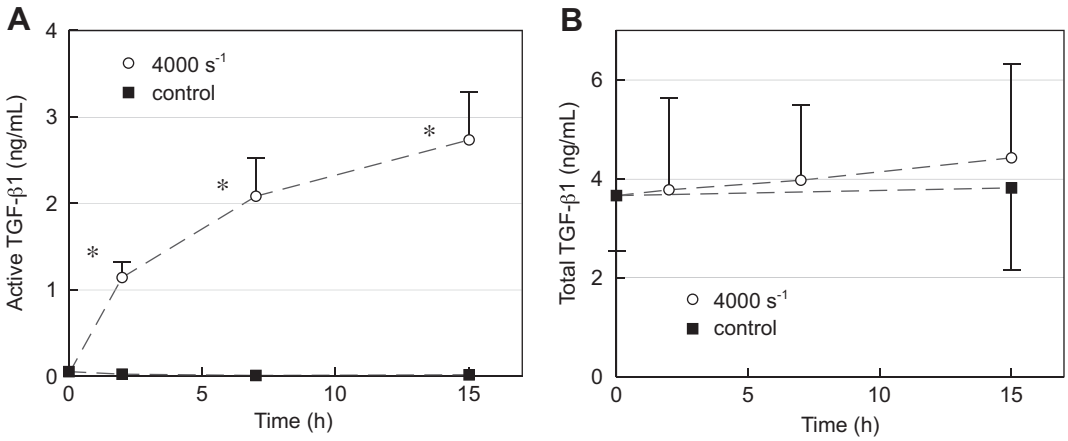

Fig. 4. Concentration of (A) active and (B) total TGF-β1 in immature bovine synovial fluid undergoing continuous circulation at 4000 s^{-1} and an unsheared control group (mean ± radius of 95% confidence interval). *$P < 0.001$ indicates statistical increase above corresponding control time point.

activate TGF-β, which may subsequently modulate the metabolic activity of the surrounding articular cartilage.

These results build upon the development of a new perspective on TGF-β activation in biological tissues which suggests that mechanical forces may play a role in addition to the traditionally characterized chemical mediators. Mechanically-induced TGF-β activation was recently observed in a series of experiments which showed that, in certain culture systems, activation requires the presence of integrins as well as a stiff cellular substrate. These observations suggested that TGF-β peptide release may result from molecular conformational changes as cells pull on the LTBP[29]. This concept of mechanical activation was further advanced in the recent study by Ahamed *et al.* which showed that mechanical shearing of blood serum can also activate TGF-β, without the intervention of integrin mediators; interestingly, this activation occurred only for the LLC, not the SLC[30]. Under continuous shearing, activation of endogenous TGF-β in serum similarly increased over time, and was more pronounced at greater shear rates. In serum, activation has been partially attributed to the shear-induced exchange of thiol-disulfide bonds mediated by platelet-derived TSP-1 [37].

The responses observed with synovial fluid share some similarities with serum, though significant differences are also observed. While the relative amount of SLC and LLC in synovial fluid has not yet been characterized, it is evident that a large fraction of SLC undergoes activation with shearing [Fig. 6(A)], unlike serum. A precise mechanism for activation in synovial fluid remains to be verified. Synovial fluid contains large amounts of hyaluronan macromolecules which undergo elongation and stretching while subjected to fluid shearing[38]; these macromolecules may become entangled with the latent TGF-β complexes. Since latency is conferred to TGF-β through non-covalent binding to the LAP protein[17], only a relatively small amount of energy conferred by such entanglements may be necessary to break this bond *via* shearing. Thus, hyaluronan-mediated molecular unfolding may play a significant role in the activation of TGF-β. An alternative mechanism whereby protease activity may be enhanced by shearing may be discounted in light of the finding that protease inhibitors do not alter the activation response (Table I).

Shear rates in synovial fluid have been calculated from estimates of the relative sliding speed (typically ~1–10 mm/s) and fluid film thickness (~10^{-3}–10^{-4} mm) between articular surfaces of a joint[31]. Therefore, peak shear rates are typically in the range of 10^3–10^5 s^{-1}, and the highest shear rate employed in this study was at the low end of this range. Therefore, physiologic joint motion may potentially activate even higher levels of TGF-β *in vivo*. Furthermore, although active concentrations of the other TGF-β isoforms were not measured in this study, it is likely that TGF-β2 and TGF-β3 are similarly activated under synovial fluid shearing, based on their similar latent configurations.

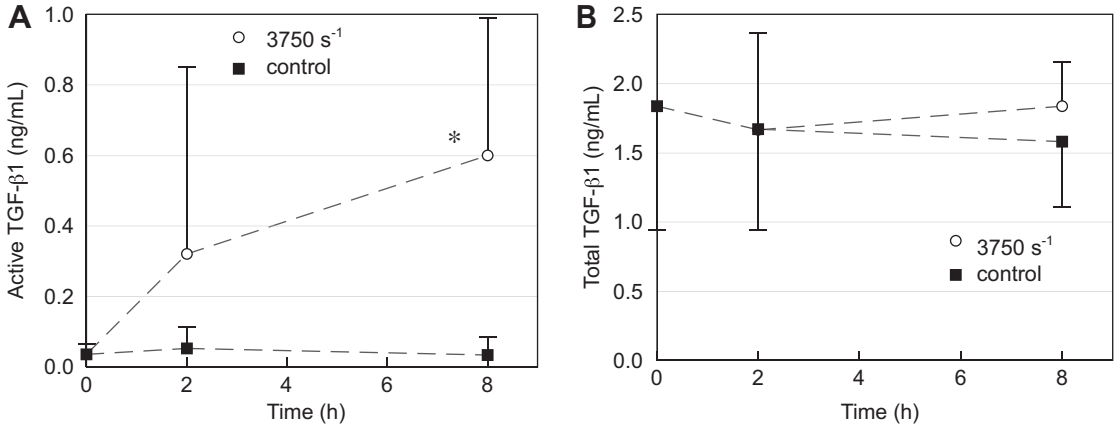

Fig. 5. Concentration of (A) active and (B) total TGF-β1 in human synovial fluid under continuous circulation at 3750 s^{-1} and an unsheared control group (mean ± radius of 95% confidence interval). *$P = 0.003$ indicates statistical increase above corresponding control time point.

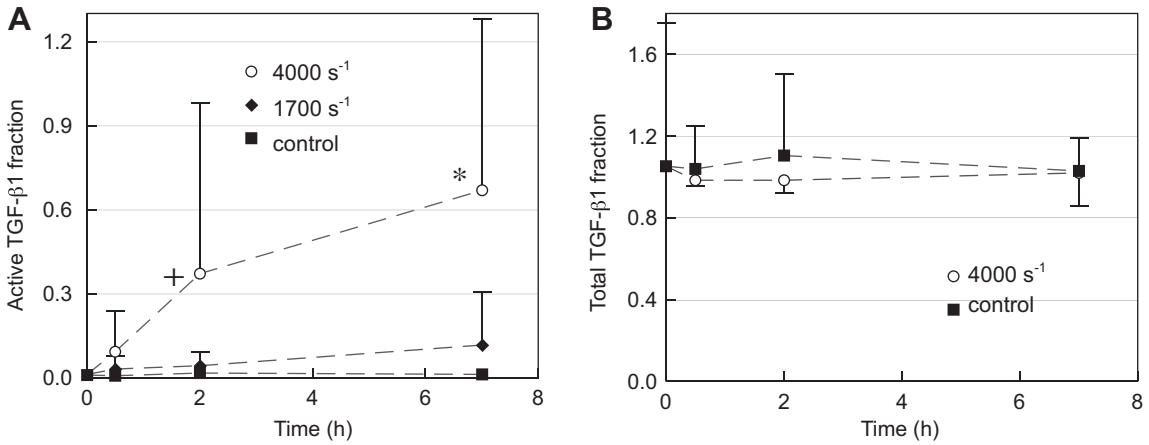

Fig. 6. Concentration of (A) active and (B) total exogenously supplemented SLC TGF-β1 in adult bovine synovial fluid undergoing continuous circulation at various shear rates. Fractions are normalized to the initial supplemented concentration (mean ± radius of 95% confidence interval). $^{*}P < 0.001$, $+P = 0.04$ indicate statistical increase above corresponding control time point.

Several studies have shown that in growth plate cartilage, MMPs released from ECM vesicles can activate ECM-bound latent TGF-β[25,26]. Interestingly, in the present study, active TGF-β was not detected in synovial fluid at any time under zero shear conditions, despite the fact that the active form is stable (Figs. 3 and 4), suggesting that protease levels in healthy synovial fluid are not sufficiently elevated for chemical activation to occur. This observation strongly suggests that shear-induced activation is the dominant mediator of TGF-β activation in healthy synovial fluid.

The shear-induced activation of TGF-β in synovial fluid is expected to have an important role on the metabolic activity of articular cartilage based on a variety of *in vivo* and *in vitro* experimental observations. Specifically, the application of exogenous active TGF-β has been shown to routinely stimulate the synthesis of DNA[39] and ECM proteins, such as proteoglycans[11,12], type II collagen[14,40], superficial zone protein[41,42], and cartilage oligomeric matrix protein (COMP)[43], while inhibiting the catabolic actions of interleukin-1 (IL-1)[10], tumor necrosis factor-alpha (TNF-α)[44], and several MMPs[45]. Additionally, the *in vivo* inhibition of native TGF-β in synovial fluid has been shown to induce loss of proteoglycans in the cartilage matrix[46,47]. Taken together, these results strongly suggest that access to TGF-β is a requisite for maintaining the biochemical content and structural integrity of healthy cartilage. In particular, due to the close proximity of sheared synovial fluid to the superficial zone chondrocytes, it is plausible that the most immediate influence of shear-activated TGF-β is on the synthesis of superficial zone protein[41]. This proximity suggests that there exists a self-regulating mechanism whereby prolonged joint sliding motion can induce a replenishment mechanism for this tribologically beneficial protein.

While the beneficial impact of active TGF-β on diarthrodial tissues is clear, it should also be noted that the *in vivo* administration of large levels of active TGF-β has been shown to induce pathological effects associated with osteoarthritis, such as inflammation, synovial fibrosis, and the formation of osteophytes[48]. Furthermore, high steady state levels of active TGF-β have been observed in pathological synovial fluid samples[7]. These results highlight the importance of the regulation of active TGF-β levels in synovial fluid in order to maintain healthy diarthrodial joint tissues. Physiologic shearing may serve this function by activating TGF-β in synovial fluid during periods of increased joint activity, and thus, inducing biochemical stimulation while mitigating pathological effects. The importance of active TGF-β regulation is further emphasized by cartilage metabolic studies that often demonstrate that biosynthesis can be optimally stimulated by intermediate doses of active TGF-β. It is interesting that these optimal active TGF-β doses are typically in the range of 1–10 ng/mL[10,14,39], similar to the concentrations activated by TGF-β shearing (Fig. 1).

Table I
Concentrations of active and total TGF-β1 in protease inhibitor supplemented and untreated control adult bovine synovial fluid samples after 7 h of continuous shearing (4000 s^{-1}; mean ± radius of 95% confidence interval)

Treatment	Active TGF-β (ng/mL)	Total TGF-β (ng/mL)
Control	1.96 ± 0.51	4.37 ± 1.13
Protease inhibitors	1.70 ± 0.31	4.56 ± 0.76

The potential physiologic role of shear-induced TGF-β activation is further supported through the demonstrated stability of activated TGF-β in synovial fluid. It should be noted that although activated TGF-β can reassociate with free LAP, the altered conformations of the activated molecules[49] make it unlikely to do so at physiologic concentration levels[50]. Regardless, the long-term stability of activated TGF-β observed in synovial fluid is an unexpected outcome, based on the traditional understanding that TGF-β has an extremely short systemic half-life due to interactions with proteases and non-specific binding proteins[33]. This stability suggests that once activated, TGF-β is able to diffuse into the surrounding articular cartilage and influence the metabolic activity of chondrocytes, especially in the superficial zone.

It has long been recognized that mechanical stimulation of diarthrodial joints is a requisite for maintaining the metabolic activity of articular cartilage. Studies have generally shown that cyclic or dynamic loading of joints enhances protein biosynthesis[51] while joint immobilization leads to degradation[52], and typically attribute these effects to a direct mechanotransduction pathway. The results of this study suggest that an additional biosynthetic effect of mechanical loading may be mediated by TGF-β activation in the synovial fluid. This mechanism would be consistent with observations that synovial fluid from animal joints subjected to exercise will enhance tissue biosynthesis beyond the levels achieved by synovial fluid from immobilized joints[53]. The results of this current investigation further suggest that these anabolic effects may be an important manifestation of shear-induced activation of latent TGF-β in synovial fluid.

Studies involving humans or animals

The Institutional Review Board of Columbia University (Morningside Campus) has determined that shear testing of human synovial fluid samples acquired from a tissue bank does not constitute human subjects research (IRB-AAAI0406).

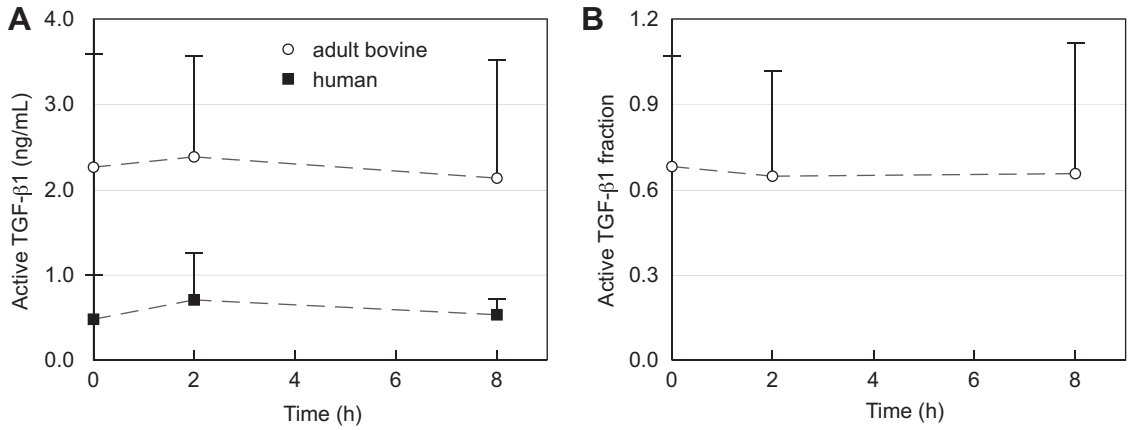

Fig. 7. Stability of shear-activated TGF-β1 levels in (A) adult bovine and human synovial fluid samples and (B) adult bovine samples exogenously supplemented with SLC TGF-β1 (mean ± radius of 95% confidence interval). Active TGF-β1 fractions in SLC supplemented samples are normalized to the initial supplemented concentration.

Author contributions

Drs. Albro, Hung and Ateshian were involved in the conceptualization of the study, forming of the hypothesis, experimental study design, and interpretation of results. Dr. Albro and Messrs. Cigan, Nims, Yeroushalmi, and Oungoulian were involved in performing the experiments and analysis of results. All authors were involved in drafting or revising the manuscript. All authors have provided their final approval of the submitted manuscript.

Role of funding source

The funding source had no involvement in the study design, collection, analysis or interpretation of data, nor in the writing of the manuscript, or the decision to submit the manuscript for publication.

Conflict of interest

The authors have no conflicts of interest to disclose with regard to this study.

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Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.joca.2012.07.006.

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